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Viral Hemorrhagic Fevers: Current Status of Endemic Disease and Strategies for Control

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The United States Army Medical Research Institute of Infectious Diseases lists four RNA viral families as the prime etiologic agents for viral hemorrhagic fevers (VHF): (1) the Arenaviridae (Argentine, Bolivian, Brazilian, and Venezuelan hemorrhagic fevers; and Lassa fever); (2) the Bunyaviridae (Hantavirus genus, Congo-Crimean hemorrhagic fever (CCHF) from the Nairovirus genus, and Rift Valley fever virus from the Phlebovirus genus) [1]; (3) the Filoviridae (Ebola and Marburg viruses); and (4) Flaviviridae (dengue and yellow fever) [2]. References 3 through 13 list the most important characteristics and classify the Arenaviridae viruses, Bunyaviridae viruses, Filoviridae viruses, and Flaviviridae viruses, respectively [3–13].

The threat posed by viral hemorrhagic fever viruses

The VHF agents pose a real threat as terror weapons for the following reasons:

1. Except for Marburg and Ebola viruses, they are widely distributed in nature
2. Many are naturally spread by airborne means

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3. Humans are widely susceptible to serious and often life-threatening infections
4. The differential diagnosis encompasses a wide variety of organisms (rickettsial disease, leptospirosis, relapsing fever, malaria, typhoid, shigellosis, sepsis, and others), making the initial recognition of a VHF virus attack difficult to distinguish
5. There is great similarity in the clinical presentations of the VHFs, making them nearly impossible to differentiate without sophisticated and time-consuming laboratory analysis
6. VHFs that respond to antiviral therapy need to be treated immediately, but clinically cannot be separated from the viral pathogens that lack specific therapy
7. Limited prophylactic and therapeutic options [14]
8. Isolation and identification of VHF agents frequently require a biosafety 4 level laboratory
9. Life-saving supportive therapy often requires an intensive care bed, which is impractical in a mass casualty situation

Fortunately, few VHFs (CCHF, Lassa fever, Andes viruses, Ebola virus, and Marburg virus) exhibit secondary human-to-human spread especially in the nosocomial setting [14–22].

This article presents the virology, pathology, clinical presentation, and control measures available for a limited number of VHF agents to assist the practitioner in the early recognition and therapeutic options for these threats.

Virology and pathology

The Arenaviridae

Arenaviruses are spherical or pleomorphic enveloped single-stranded bi-segmented RNA ambisense viruses that use virion RNA-dependent RNA polymerase for replication. The first arenavirus was isolated in 1933 (the lymphocytic choriomeningitis virus), and the first arenavirus hemorrhagic fever virus was isolated in 1958 (Junin virus, the cause of Argentine hemorrhagic fever) [3,23,24]. Machupo virus was isolated from cases of Bolivian hemorrhagic fever in 1965, and Lassa virus was isolated in 1970 [3]. There are 18 arenavirus species, with at least seven causing hemorrhagic fever: (1) Lassa fever virus–Lassa fever, (2) Junin virus–Argentine hemorrhagic fever, (3) Machupo virus–Bolivian hemorrhagic fever, (4) Guanarito virus–Venezuelan hemorrhagic fever, (5) Sabia virus–hemorrhagic fever with extensive hepatic necrosis, (6) Whitewater Arroyo virus–hemorrhagic fever with liver failure, and (7) Oliveros virus–hemorrhagic fever [19,25,26].

Arenaviruses cause chronic asymptomatic infection in rodents. Persistent rodent infection is caused by both molecular mechanisms and failure of the host's immune system. Congenital neonatal infection results in high-titer life-long viral infection and abundant urinary excretion (up to 10^5 plaque-forming

units per milliliter urine). During the acute infection, there is no cytopathic effect and most rodent cells remain infected for life [19,27].

Humans and nonreservoir hosts inhale aerosols containing the virus. The virus has been shown to enter by gastrointestinal and respiratory epithelial cells through the apical plasma membrane [24,28]. The cellular receptor for the Old World arenaviruses Lassa fever virus and lymphocytic choriomeningitis virus seems to be α -dystroglycan. α -Dystroglycan is a cell surface receptor that is the link between the extracellular matrix and the actin-based cytoskeleton [29].

After attachment, there is local viral replication. The virus spreads to hilar lymph nodes, lung, and other organs. There is no pulmonary consolidation, but interstitial infiltrates and edema do occur. Initially, macrophages are infected. This is followed by widespread epithelial involvement [19,30].

In fatal cases, fulminant viremia is believed to be caused by failure or delay in the cellular immune response. The pathogenicity of Lassa virus is related to its resistance to interferon. In one study, however, the interferon sensitivity of the Lassa virus isolate did not correlate with its lethality [31].

Pichinde virus, because it does not infect humans, has been used in the guinea pig model to mimic human Lassa fever [19]; 7 days after infection, during the initial viremia, there is weight loss (up to 25%) and fever. Macrophages are the cells that are primarily infected. Epithelial cells then become infected with little involvement of endothelial cells. Focal necrosis of the liver and adrenal glands, mild interstitial pneumonitis, marginal zone necrosis in the splenic white pulp, and intestinal villous blunting are seen histologically. The guinea pigs develop progressive decreasing cardiac output not caused by carditis, but from release of soluble inflammatory mediators (ie, tumor necrosis factor- α) [19].

In the Balb/c neonatal mouse model, Pichinde viral infection is fatal in most mice. In surviving mice, the virus was gradually cleared but could be detected for up to 9 months in the kidneys and brain. The animals had high antibody titers and major histocompatibility group restricted cytotoxic T-cell activity. Pichinde virus experiments require a biosafety level 2 laboratory [32].

Pirital virus coexists in the same region of Venezuela as Guanarito virus (the cause of Venezuelan hemorrhagic fever) and was originally isolated from the cotton rat (*Sigmodon alstoni*) [33]; this virus provides another model that mimics fatal human Lassa fever infection in Syrian golden hamsters (*Mesocricetus auratus*). The golden hamsters develop interstitial pneumonitis, splenic lymphoid depletion and necrosis, and multifocal hepatic necrosis without a great deal of inflammatory infiltration. Special staining with in situ terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate (dUTP) nick-end labeling stain demonstrates hepatocytes undergoing apoptosis or necrapoptosis. This model requires a biosafety level 3 laboratory [34].

South American hemorrhagic fever patients develop bleeding secondary to severe thrombocytopenia, caused in part by interferon-induced maturation

arrest of megakaryocytes. Lassa fever is more likely to cause hepatic failure than thrombocytopenia. Risk of death from Lassa fever is correlated with high aspartate transaminase serum levels [19].

The Bunyaviridae

The Bunyaviridae are a family of animal and plant viruses consisting of 51 species (47 definite and 4 tentative), divided into five genera; four genera infect animals and one infects plants [4–8,35,36]. The virus was first isolated from *Aedes* mosquitoes in 1943 during a yellow fever epidemic [36].

The Bunyaviridae are spherical enveloped viruses (80–120 nm in diameter) [4]. The genome consists of a large (designated L), medium (M), and small (S) single-stranded negative-sense RNA with the same complementary nucleotides at the 3' and 5' ends. Within each genus, the terminal nucleotide sequence is conserved, but differs significantly from genus to genus within the Bunyaviridae family. All members of the family contain viral sense RNA, whereas Phlebovirus and Tospovirus genera also contain complementary sense RNA. Bunyavirus, Hantavirus, and Nairovirus genera use negative-sense coding, whereas Phlebovirus (includes Rift Valley fever) and Tospovirus (plant diseases) genera use ambisense coding [35].

Bunyavirus genus

Bunyavirus pathology is exemplified by that of California encephalitis, La Crosse, and Jamestown Canyon viruses. The cycle begins with an asymptomatic amplifying infection in natural vertebrate hosts (adult chipmunks, squirrels, foxes, and woodchucks for La Crosse virus; white-tailed deer for Jamestown virus; and snowshoe hares for snowshoe hare virus). Mosquitoes (ie, *Aedes triseriatus* in La Crosse virus, *Culiseta inornata* in Jamestown virus) become infected after feeding on these animals. The virus replicates in the mosquito midgut, then disseminates to all organs including the ovaries and mosquito salivary gland. The mosquito bite, in turn, infects humans. Mosquito ovarian infection is important in maintaining the virus in the mosquito population.

In humans, virus spreads from subcutaneous tissue to skeletal muscle where it replicates, and then secondarily spreads by lymphatics to distant skeletal and cardiac muscle where another round of replication takes place. From here, the virus disseminates to the central nervous system where it replicates in neurons and glial cells. This is followed by neuronal cell necrosis. Death occurs in 3 to 4 days. Brain lesions (principally the cerebral cortex and brainstem) consist of edema, perivascular cuffing, glial nodules, and leptomeningitis with some areas of focal necrosis [36].

Phlebovirus genus

In 1908, Doerr demonstrated that phlebotomus fevers were caused by a filterable agent [7]. The Phlebovirus that causes Rift Valley fever was first

isolated from a newborn lamb in 1930 during an epidemic in sheep that caused both abortions and high mortality. "Sandfly fever" Sicilian and Naples viruses were isolated from American troops in Palermo in 1943 and Naples in 1944. Similar diseases were described during the Napoleonic Wars and again in 1905.

"Abortion storms" were described as early as 1931 as Rift Valley fever swept through herds of cattle and sheep. More recent abortion storms have been attributed to equine herpesvirus type 1 (especially Army 183 F–fetal strain); *Coxiella burnetii*; Thogoto virus (transmitted by Ixodid ticks); *Neospora caninum*; and one strain of equine arteritis virus [37–44]. It is important to be aware that abortion storms may be an early sign of a zootic or a bioterrorist attack with a hemorrhagic fever virus or other agents. Unfortunately, only 25% of abortion storm etiologic agents are ever identified [45].

Rift Valley fever infection is initiated by the bite of an infected mosquito. The inoculated virus is trapped in the local lymph nodes where it replicates and becomes the source for the primary viremia. The major organs are infected, and the second round of replication takes place in distant lymph nodes, spleen, liver, adrenals, lungs, and kidneys. Necrotic foci develop in the liver and in the brain of patients with clinical encephalitis. In hemorrhagic fever, thrombocytopenia and fibrin deposits in major organs are seen [36].

Punta toro virus infection in hamsters has become an experimental model for Rift Valley fever. In this model, the liver injury is responsible for the hemorrhagic complications. Viral replication directly causes apoptosis. Cellular viability decreases 12 hours after infection. Caspases 3/7 are activated, phosphatidylserine translocation and DNA fragmentation occur between 48 to 72 hours. Viral infection alone without systemic inflammatory reaction induces the hemorrhagic disease and suggests targets for therapeutics [46].

Nairovirus genus

The Nairovirus genus consist of tick-borne viruses with few being transmitted by culicoides flies and mosquitoes (CCHF: ticks [some species of *Hyalomma*, *Dermacentor*, and *Rhipicephalus*] and flies; Nairobi sheep disease: ticks, flies, mosquitoes) [36]. CCHF worldwide distribution mirrors *Hyalomma* ticks more closely than other species. The disease has been reported throughout sub-Saharan Africa, South Africa, Madagascar, the Middle East, European Russia, Pakistan, Afghanistan, the central Asian republics, Bulgaria, the former Yugoslavia, northern Greece, and Xijiang province of northern China [16,36,47]. CCHF and nairoviruses survive through various tick life-stages (transstadial transmission) and from tick generation to generation (transovarian transmission). Vertebrates (including ground-feeding birds in European Russia, Bulgaria, and Greece) provide blood-meals for infected ticks, become infected, and the viral source for uninfected ticks (viral amplification) [16,36,47]. Sheep, goats, cattle, ostriches, large wild herbivores, hares, and hedgehogs are known to amplify CCHF, and sheep and goats are known to amplify the other nairoviruses.

In Africa, the distribution of nairovirus infection follows the distribution of the tick host, *Rhipicephalus appendiculatus*. In the Middle East and India, the distribution of human infection follows the *Haemaphysalis intermedia* ticks and the closely related Ganjam virus [36].

Nairovirus reproduces in spleen, liver, and kidneys; and in sheep, goat, and suckling mouse infections, the vascular endothelium becomes the primary viral target. Edema and necrosis of the capillary walls of the mucosa of the intestine, gallbladder, and female genital tract is seen with the development of hemorrhage and inflammation [36]. In humans, local viral replication follows spread by blood and lymphatics to all major organs, especially the liver. There is edema, hemorrhage, and necrosis; diffuse intravascular coagulation (DIC); and thrombocytopenia with the expected abnormalities of the coagulation profile. IgM and IgG responses are detectable by Day 9 in survivors and are absent in most fatal cases [36].

Hantavirus genus

The *Hantavirus* genus first came to medical attention in 1934 with the publication of a case of hemorrhagic fever with renal syndrome [5]. Between 1951 and 1953, 3000 cases of an acute febrile illness, 33% with hemorrhagic manifestations, were reported among the United Nation's troops during the Korean War. First known as Korean hemorrhagic fever, now it is commonly referred to as hemorrhagic fever with renal syndrome (HFRS) [36]. *Apodemus agrarius* (the field mouse) was found to be the reservoir. The virus, first isolated in 1978, was designated the Hantaan virus after the Hantaan River (Korea) where many of the original cases were described. *Rattus norvegicus* and *R. rattus* were the hosts of Seoul virus, the cause of urban HFRS [5,36]. In Scandinavia, nephropathia epidemica, a renal disease with mild or without hemorrhagic manifestations, was caused by Puumala virus spread from *Clethrionomys glareolus*, the bank vole. The only insectivore to harbor a hantavirus is the *Suncus murinus*, the Indian tree shrew. Prospect Hill virus was the first hantavirus causing human disease to be described in the Americas (*Microtus pennsylvanicus* [meadow vole] host). Severe HFRS of the Balkans was caused by Dobrava virus associated with *Apodemus flavicollis*. At least three subfamilies and 28 species of hantaviruses cause either HFRS or hantavirus pulmonary syndrome (HPS) [5,36,48].

In 1993, in the southwestern United States (Four Corners region, the meeting point of New Mexico, Arizona, Colorado, and Utah) an outbreak of an influenza-type illness that rapidly progressed to respiratory failure, shock, and death in 2 to 10 days (HPS) was found to be caused by the hantavirus Sin Nombre, with a reservoir in the deer mouse, *Peromyscus maniculatus* [49]. In Argentina, *Akodon azarae* is the most abundant rodent with a hantavirus seroprevalence of 9.3% [27].

The first outbreak of HPS in Central America (Los Santos, Panama) occurred in 1999 to 2000 with 11 cases, nine confirmed by serology and three fatalities. Household and neighborhood serologic studies found seropositivity

of 13%, but no suggestion of person-to-person spread [50]. Western Venezuela is the home of the Maporal virus (closely related to South American HPS viruses) that infects the fulvous pygmy rice rat (*Oligoryzomys fulvescens*) [51].

Since that discovery, 10 different hantaviruses with 10 different rodent reservoirs have been shown to cause HPS throughout North and South America [5,36]. Identification of high-risk areas is essential for controlling human infection. Bayou virus is the second leading cause of HPS in the United States. Its rodent hosts include *Oryzomys palustris* (most commonly infected species, 16% seroprevalence rate), *Sigmodon hispidus*, *Peromyscus leucopus*, *Reithrodontomys fulvescens*, and *Baiomys taylori*. The heaviest male rodents had the highest seroprevalence. Seroprevalence is higher in the coastal prairie (20%) than old-fields (10.5%), and is directly related to host population densities [52].

The first cases of HPS in Maranhao State, Brazil, have been identified and the viruses designated as the Anajatuba and the Rio Mamore viruses (isolated from *Oligoryzomys fornesi* and *Gikicgukys scuyreys*) [53,54]. Two cases of HPS appeared in two areas of central Bolivia after dense forest was destroyed and replaced with pastures and sugarcane. *Oligoryzomys microtis* and *Calomys callosus* were identified as the rodent reservoirs associated with human disease [55]. It is interesting to note that neither HPS nor HFRS have been reported in Australia despite the fact that Hantavirus antibody-positive rodents have been found across the continent [56].

Hantavirus establishes an asymptomatic infection in its natural rodent reservoir that persists for months or years. In at-risk areas, the disease is spread (horizontally) by scratching, biting, or infected aerosols among the rodents. The virus spreads to all organs but particularly concentrates in the kidneys and lungs, with high rates of replication in the salivary glands. Virus is shed in urine, feces, and saliva, peaking 2 to 10 weeks after infection, and continuing for life, although in reduced quantity with rising antibody titers. There is no cell death observed in the infected animal-hosts. Seroprevalence studies in endemic areas have found *R norvegicus* to be the most frequently infected species (Kinmen study: *R norvegicus*, 50%; *Mus musculus*, 20%; *R flavipectus*, 2%) [57].

Most hantavirus infections are asymptomatic or go undetected. In India, there have been no reports of either HPS or HFRS [58]. A study of 152 patients with febrile illness found 23 (14.7%) seropositive by enzyme immunoassay for IgM directed against hantavirus. Eighteen (82%) of 22 patients were positive by indirect immunofluorescence assay. In the same study, 5.7% of healthy blood donors were positive by enzyme immunoassay, and 40% of these patients were positive by immunofluorescence assay [58]. A similar survey on Kinmen (an island between China and Taiwan) found a seroprevalence of 8.23% among scrub typhus-negative individuals [57].

HPS and HFRS infect humans after inhalation of infected rodent excreta. There is no cytopathologic effect in infected human cells. The lethal pathology seems to be immune modulated and a direct result of viral

induction of cell apoptosis [29,46,59]. Other Bunyaviridae infections (Akabane and Aino viruses, the causes of abortion, stillbirths, and congenital defects in cattle, sheep, and goats) also result in the induction of apoptosis [60].

Paradoxically, serologic tests on individuals with frequent occupational contact with rodents had little or no evidence of exposure to Sin Nombre virus (the cause of HPS); Whitewater Arroyo virus (an Arenaviridae); or Ampari virus [61].

HFRS patients have acute tubulointerstitial nephritis. Puumala virus–associated HFRS results in interstitial infiltration of lymphocytes, plasma cells, monocyte-macrophages, neutrophils, and eosinophilic granulocytes. In HPS and HFRS there is a general increased production of cytokines [36].

Monocytes and macrophages play an important role in HPS and HFRS pathophysiology. Viral infection causes HFRS-infected cells to undergo complex metabolic changes with a resultant (infected cell) increase in bactericidal activity against *Staphylococcus aureus* [62].

The Filoviridae

Ebola and Marburg make up the two genera of the filoviridae [9,10,63]. Both genera are enveloped, single-stranded negative-sense RNA viruses. Ebola is bacilliform, bent-pin shaped; Marburg is filamentous shaped. Ebola virus was first isolated and identified in 1976, with its genetic sequence published in 1989. Marburg virus was first described in 1967, and its gene sequence was published in 1992 [9,10]. There are four species of Ebola virus (Zaire, Sudan, Reston, and Cote d'Ivoire) and one species of Marburg virus [63,64].

The macaque develops disease similar to humans after inhalation or injection of the virus. The Zaire species is the most virulent (60%–90% case fatality rate [65,66]) and the Reston species is the least virulent. The viral infection produces viral cytopathology, cytokine-mediated vascular leaks, and impairment of the host response. Macrophages and monocytes are some of the earliest cells infected, damaging the host's immune response and facilitating the spread of the virus.

Human pathology

Patients dying of either Ebola or Marburg hemorrhagic fevers exhibit necrosis of parenchymal cells of the liver, spleen, kidneys, ovaries, and testes. In the liver, there is hepatocellular necrosis with intact, hyalinized, ghost-like cells amid cellular debris filled with virions. Intact hepatocytes display intracytoplasmic inclusion bodies, which represent aggregates of viral nucleocapsids. There is injury to the microvasculature (Kupffer cells and capillary endothelium) with increased endothelial permeability that contributes to the shock and bleeding. There is lymphoid depletion in the spleen and lymph nodes with vascular follicular necrosis [64].

In fatal cases, there is no antibody response. In survivors and patients with a protracted course, the patients develop a delayed humoral response

[16]. Peters and coworkers [66] provide a detailed summary of the pathologic effects of the filoviruses. The viruses suppress the induction of interferon by VP35 protein, and block interferon action by VP24 protein [67].

Transmission of disease is person-to-person. Aerosol spread to nonhuman primates has been documented. It is believed that droplet spread is the common person-to-person vehicle among infected humans; aerosol spread is probably rare. Handling infected monkeys has been implicated in some cases. The source in nature remains unknown, although recent evidence of asymptomatic infection in fruit bats has been reported [64,65,68].

The Flaviviridae

In 1900 in Cuba, Walter Reed and James Carroll of the US Army Medical Corp proved Dr. Carlos Finlay's theory that yellow fever was transmitted by mosquitoes. Yellow fever virus was the first flavivirus to be isolated (1927) and grown in vitro (1932). There are 73 species of flaviviruses. The Flaviviridae are spherical, enveloped, single-stranded positive-sense RNA viruses [11–13,69–71]. The Flaviviridae consist of three genera: (1) Flavivirus (dengue virus, Ilheus virus, Japanese encephalitis virus, Kyasanur Forest disease virus, Kunjin virus, louping ill virus, Murray Valley encephalitis virus, Omsk hemorrhagic fever virus, Powassan virus, Rocio virus, St Louis encephalitis, tick-borne encephalitis, Wesselsbron disease, West Nile fever, yellow fever, and Zika disease); (2) Hepacivirus (hepatitis C virus); and (3) Pestivirus (Bovine viral diarrhea viruses, border disease virus, classical swine fever virus, and various animal pestiviruses) [11–13,72,73].

Mosquito-borne flavivirus hemorrhagic fevers

Natural transmission of flaviviruses may take place by transfer of virus from host to host by contaminated mouthparts of mosquitoes. More likely, either the arthropod or mosquito acquires the virus through a blood meal; the virus replicates in the epithelial cells lining the mesenteron (midgut); the replicated virus escapes to the hemocele and infects the salivary gland; and is secreted in the saliva during the next feeding, infecting a new host [70,74]. With dengue viruses, the virus may enter a number of mosquito ova, directly infecting mosquito progeny.

Dengue virus

Dengue virus survives in two hosts: the vector and the reservoir. *A aegypti* and other *Aedes* species are the most common mosquito vectors. *A aegypti* requires 10^5 /mL viral titers in humans to become infected after a blood meal. This helps select out for strains of virus that rapidly reproduce [70,74].

In nonhuman primates, the virus replicates principally in monocytes in skin, lymph nodes, spleen, liver, lung, and thymus. Dengue fever is self-limited with skin lesions showing swelling of endothelial cells of small vessels,

perivascular edema, and monocyte infiltration. Dengue hemorrhagic fever is the result of sequential infection. The primary pathophysiologic change in dengue hemorrhagic fever is vascular permeability, leakage of plasma into the extravascular compartment, hemoconcentration, and hypotension.

It is believed that immune reaction to a second infection with dengue virus results in dengue hemorrhagic fever. Pre-existing antibodies to dengue virus are heterologous, do not neutralize the virus, and permit the virus to replicate freely in the macrophage. A second theory contends that the virus mutates [70,74].

Chimeric dengue, tick-borne encephalitis, and West Nile virus (live-attenuated) vaccines are now under development [75]. Other control methods under consideration have been the introduction of sterile male mosquitoes to facilitate the displacement of the insect from the habitat [76].

Yellow fever virus

Yellow fever virus is transmitted to humans from infected humans or infected primates by tree-hole breeding mosquitoes (*Haemagogus janthinomys*, *Haemagogus* sp, *Sabethes chloropterus*, *Aedes* sp) usually during the tropical wet season and early dry season. As with other hemorrhagic fever viruses, the mosquito introduces the virus into the skin where it replicates locally and then spreads into the regional lymph nodes. The virus then disseminates by the bloodstream to the liver, spleen, bone marrow, and myocardium. The hallmark of the disease is steatosis, apoptosis, and necrosis mainly in the midzonal region of the liver [77]. Kupffer cells are infected followed by hepatocytes in the midzone of the liver. Midzonal necrosis develops. The appearance of Councilman's bodies is indicative of hepatocyte apoptosis with minimum mononuclear infiltrates [78]. There is a predominance of apoptosis over necrosis with contributions from a variety of activated lymphocytes and various cytokines, notably transforming growth factor- β [77].

Yellow fever vaccine is a live-attenuated vaccine given every 10 years for those at risk and produces immunity in 95% of recipients [79,80]. Experimental compound ZX-2401 inhibits yellow fever virus, dengue virus, bovine viral diarrhea virus, banzi virus, and West Nile virus and may become a candidate chemotherapeutic agent [81].

Tick-borne flavivirus hemorrhagic fevers

Kyasanur Forest disease (India). In humans, the virus produces parenchymal degeneration of the liver and kidneys, hemorrhagic pneumonia, increase in the reticuloendothelial tissue in the liver and spleen, and marked erythrophagocytosis. Macaques develop fatal infections with lymph tissue necrosis and some primates develop encephalitis with chromatolysis of neurons and focal demyelination.

The primary vector is the *Haemaphysalis spinigera* tick, although 10 species of ixodid ticks have also been implicated. Ticks transmit the virus among

themselves by transstadial and transovarial transmission. Bats and ground-dwelling birds may be reservoirs. Cows, goats, and sheep have also become infected, but their epidemiologic importance is unknown [70,82].

Omsk hemorrhagic fever (Siberia). *Dermacentor reticulatus* and *Ixodes apronophorus* are suspected vectors, and the *Arvicola terrestris* is believed to be the host [70].

Clinical presentations and management

Managing VHF involves basic diagnostic, therapeutic, prophylactic, and infection control concepts, and a practical monitoring system for contacts. Guidelines must be understandable, practical, and make clear recommendations concerning any controversial treatments [17,83]. It is recommended that these guidelines include the following for possible VHF bioterrorism incidents:

1. Identify the patient and develop a complete differential diagnosis (which most often includes malaria, typhoid, gastroenteritis, meningococemia, or other diseases). Examples of case definitions may be found on page 248 of reference 84 [84].
2. Notify public health authorities (in the United States and its territories, this is done through the local or state health departments).
3. Clinical laboratories must be organized and prepared through quality assurance activities properly to handle, test, and forward when indicated suspect specimens [85]. Confirm or eliminate the diagnosis by either viral isolation, identification of viral antigens by ELISA or polymerase chain reaction (PCR), or use of the modern antibody testing. All viral isolation specimens should be sent to a biosafety level 4 laboratory.
4. Isolate the patient and institute and strictly enforce infection control measures (strict isolation, negative pressure rooms). Intensive care is likely to be required. Adequate numbers of isolation beds where an “intensive” level of care may be delivered should be preplanned [86,87]. Details of the 11 specific protective measures recommended to prevent nosocomial spread of VHF viruses (including the use of the N-95 mask or powered air-purifying respirators) may be found in reference 14, page 210 [14]. Special training is necessary for laboratory workers handling patient specimens, postmortem practices, and environmental decontamination (linens, beds, hospital rooms, and so forth).
5. Identify at-risk contacts and institute prophylaxis where available and if recommended by public health authorities. At the present time, ribavirin prophylaxis is controversial [14].
6. On-going monitoring must be instituted for all at-risk contacts. A self-monitoring system is preferred because it is less labor- and resource-intensive [83].
7. Aggressively manage the patient with supportive therapy.

8. Provide specific antiviral therapy where available. Begin ribavirin pending the identification of the etiologic agent. Continue treatment if an arenavirus or a bunyavirus is identified. Discontinue therapy if flavivirus or filovirus or other viruses that do not respond to ribavirin are identified [14].
9. There is no licensed vaccine except for Yellow fever. Ribavirin prophylaxis is controversial because it may only delay the onset of disease [14].

The Arenaviridae

Lassa virus: Lassa fever

Lassa fever virus, of the Arenaviridae [88], was discovered 30 years ago and is endemic in West equatorial Africa. Serosurveys reveal antibody prevalence of 8% to 52% in Sierra Leone, 4% to 55% in Guinea, and 21% in Nigeria [89]. In Sierra Leone, Guinea, and Nigeria, the United Nations Development Program estimates that there are 59 million seronegative at-risk individuals, 3 million first-time infections per year, 3 million reinfections per year, and 67,000 deaths per year [89]. The *Matomys natalensis* rodent is its principle host in savannah and forest regions (11% antibody positive) and *Mus musculus* its principle host in coastal and urban areas (5% antibody positive) [90]. *M natalensis* caught near homes are 0% to 80% seropositive. Five percent to 22% of susceptible individuals seroconvert each year; the ratio of illness to infection is 9% to 26%; and 5% to 14% of those who seroconvert is febrile. Mortality rates are estimated at 1% to 2% of all those infected [91].

The peak incidence is believed to be in the dry season (January–March), but in Sierra Leone it overlaps with the rainy season (May–November). The incidence of disease in tropical endemic regions may drop off into the rainy season because of difficulty with travel [89]. The virus causes 5000 deaths among the 100,000 to 300,000 people per year it infects. Most infections are mild or asymptomatic (80%) and but there is a 1% overall mortality and a 15% to 20% mortality for patients requiring hospitalization.

Lassa fever may present anywhere in the world that is accessible to air travel [89,92,93]. Guidelines need to be clear and practical; international aspects need to be considered including the need for “reliable risk assessment to be performed before patients are medically evacuated”; and self-monitoring should be considered, because active surveillance is resource intense [83].

Although the incubation period is from 5 days to 3 weeks, most cases present within 7 to 14 days after exposure. The onset of the disease is gradual with fever, malaise, and myalgia. The patient develops conjunctival injection, pharyngitis with white and yellow exudates or ulcers, cough, chest pain, and abdominal pain with nausea and vomiting. Patients with mild disease improve within 10 days.

Severely ill patients develop facial and laryngeal edema, cyanosis, mild bleeding, and shock. In some patients severe disease is commonly complicated by pleural and pericardial effusions. These patients are noted to

have mild thrombocytopenia and dysfunctional platelets. The patients' white blood cell counts are normal or reduced, and commonly with Lassa fever there is a mild elevation of aspartate transaminase levels. In some patients hepatitis is severe [16,17].

Thirty percent of patients develop permanent late sensorineural deafness. Sudden onset of deafness has been associated with Lassa virus seropositivity. There is no relationship between severity of illness, initial hearing loss, and eventual recovery [89]. Patients with neurologic complications (tremors, confusion, seizures, and coma) often die.

Pregnant women have the highest mortality rates (16%). Lassa virus crosses the placenta and commonly results in abortion, particularly in the third trimester. Overall mortality is between 1% and 2%. In hospitalized patients, the mortality is 15% to 20%. Poor prognostic signs include pharyngitis, tachypnea, bloody diarrhea, and high fever.

In children, 100% have fever and 60% have a cough and vomiting. The highest overall prevalence is among 5- to 9-year-old age group (41%). More girls develop clinical disease [94].

Differential diagnosis of Lassa hemorrhagic fever includes malaria, typhoid, other VHF, meningococemia, and sepsis. In Sierra Leone, fever, pharyngitis with exudates or ulcers, chest pain, and proteinuria were likely to be Lassa fever in 80% of cases [16,17]. On admission, most patients have antibodies against the virus (53% IgG and 67% IgM). Positive ELISAs for Lassa virus antigen combined with IgM antibody is 88% sensitive and 90% specific for the diagnosis of acute Lassa fever [89].

Lassa fever is acquired by rodent excreta that is either inhaled or contaminates food, or by person-to-person transmission. It is essential that precautions be taken with those who recover from the disease, because viremia is present into the second week of the clinical illness and virus is found in urine for 3 to 9 weeks and in semen for 2 to 3 months. There are no data as to the relative risk of sexual transmission [89,92].

Diagnosis is made by isolating the virus from blood, throat swabs, or urine in biosafety level 4 laboratory. ELISA detects Lassa virus antigen that may be confirmed by reverse transcriptase (RT)-PCR by Day 3 of illness. Specific antibody testing by IgM ELISA is also available and has replaced the indirect fluorescent antibody tests [16,89]. Other laboratory abnormalities include lymphocytopenia and thrombocytopenia that peaks between 10 and 11 days [89].

For prophylaxis and treatment of Lassa fever including pregnant women [14,16,17,84,87,95], high-dose intravenous ribavirin is recommended, 2 g intravenous loading dose followed by 1 g intravenously every 6 hours for 4 days. This is followed by 0.5 g intravenously every 8 hours for 6 days. It is recommended that women stop breast-feeding. Another recommended intravenous regimen is an initial dose of 30 mg/kg followed by 15 mg/kg every 6 hours for 4 days, followed by 7.5 mg/kg every 8 hours for 6 days. Oral ribavirin doses are, 2 g loading dose followed by 4 g/d in four divided doses

for 4 days followed by 2 g/d for six doses. Oral ribavirin is believed to be only half as effective as intravenous therapy. Intravenous ribavirin, if given within 6 days of the beginning of symptoms, reduces mortality by 90% [89]. Postexposure prophylaxis with ribavirin is recommended by some but not all authorities. This consists of 2 g orally in four divided doses for 7 days [14,16,17,78,84,95]. Zidampidine, a derivative of zidovudine, is a new agent that seems effective in treating the CD-1 mouse model of Lassa VHF [96].

Control of rodents in or near dwellings and strict isolation of hospitalized patients are important [97]. In one survey in Ekpom, Nigeria, of 218 captured *M natalensis* rodents, 46.8% were positive for complement fixing antibody to Lassa virus [98].

New vaccines, including a DNA mini-gene vaccine that encodes for Lassa virus proteins (full-length Lassa nucleoprotein) has been found to induce CD8⁺ T-cell responses in mice that can protect against lymphocytic choriomeningitis virus and Pichinde virus. A DNA vaccine that encodes for a nine amino acid sequence from Lassa nucleoprotein has also induced CD8⁺ T cells and has been protective in the mouse model against viral challenges [99].

Another candidate vaccine has been produced from an attenuated recombinant vesicular stomatitis virus that expresses Lassa viral glycoprotein. The vaccine protected nonhuman primates against a lethal viral challenge, and none of the test animals was found to shed the virus. Both protective humoral and cellular responses occurred. Despite a documented Lassa fever virus viremia 7 days after the challenge, none of the vaccinated animals displayed evidence of clinical disease [100].

A live attenuated vaccine produced from the reassortment of genomic segments from Lassa virus and Mopeia virus encodes for major viral antigens (nucleocapsid and glycoprotein of Lassa virus and RNA polymerase and zinc-binding protein of Mopeia virus). Immunity against Lassa virus has been demonstrated in guinea pigs, mice, and Rhesus macaques. Two of the monkeys were examined and revealed no histologic lesions or signs of disease [101]. The nonpathogen, Uukuniemi virus (a Bunyaviridae), has been used as a model for more than 30 years to study the molecular and cell biology of the highly pathogenic members of this family [102,103].

Approximately 20 cases of Lassa fever imported into western countries have resulted in no secondary clinical cases. The contacts of an imported case from the Ivory Coast by way of Lisbon to Germany were studied. The patient was diagnosed by PCR and died of hemorrhagic fever by Day 14. Of 232 contacts, 149 (with 30 close contacts) were tested serologically. No clinical illness was reported, and only a physician who examined the patient on Day 9 was IgG antibody positive. Ribavirin (10 mg/kg orally four times daily for 5–8 days) prophylaxis was started by 16 of the high-risk and close contacts after the index case's diagnosis was confirmed. Eleven of these patients had reversible increases in bilirubin (one person with jaundice on Day 4 of therapy), and nine had decreases in hemoglobin [104].

Recommendations for the management of Lassa fever in Europe [105] include the following. Management of patients has varied. Negative pressure rooms and universal precautions (The Netherlands), completely enclosed “plastic bubble units” (Trexler Units, United Kingdom), and staff isolation suits (Germany) have all been used. The Trexler Units made it impossible to provide intensive care to the sickest patients, and the isolation suits made it difficult for staff to care for patients more than 3 hours at a time. One publication concluded that all patients should be hospitalized; mildly ill patients may be managed with negative pressure rooms and strict isolation. Patients more severely ill should be managed in high-security isolation facilities [105].

Low-risk contact monitoring should consist of self-monitoring of temperatures without reporting regularly to health authorities. Persons directly in contact with patients’ body fluids, blood, or secretions should self-monitor temperatures and report regularly to the health departments [105].

Evidence of the efficacy of ribavirin prophylaxis is limited. Ideal dose is 1 g by mouth per day in two divided doses. In Germany and the United Kingdom, prophylaxis is offered to high-risk contacts only. It is recommended that contacts be informed of the adverse reactions and be offered the medication [105].

Guanarito virus: Venezuelan hemorrhagic fever

The virus for Venezuelan hemorrhagic fever was first described in 1990. The natural host is the cane mouse, *Zygodontomys brevicaudus*. The clinical presentation is similar to Argentine hemorrhagic fever (fever, thrombocytopenia, bleeding, and in some patients neurologic complications), with 7- to 14-day incubation and 10% to 16% mortality [16,17]. Treatment and prophylaxis are the same as for Lassa fever [16,17]. Convalescent serum therapy may also be effective [97]. Diagnosis is made by isolating the virus in a biosafety level 4 laboratory. Real-time PCR assays are under development [106].

Sabia virus: Sabia virus hemorrhagic fever

Sabia virus hemorrhagic fever is characterized by marked liver necrosis and may be mistaken for yellow fever [107]. A case has been successfully treated with ribavirin. Treatment and prophylaxis are the same as for Lassa fever [16,17,95]. Rodent control in or near dwellings is important [97]. Diagnosis is made by isolating the virus in a biosafety level 4 laboratory. Real-time PCR assays are under development [106].

Junin virus: Argentine hemorrhagic fever

As with Lassa fever, patients with Argentine hemorrhagic fever present with a nonspecific illness. In 3 to 4 days, they become extremely ill with hypotension, petechiae in the soft palate, axilla, and gingiva.

Neurologic findings are more common than in Lassa fever. They begin on the fourth day of the illness with the onset of hemorrhage. Patients are more

irritable, lethargic, and display muscular hypotonia, hyporeflexia, areflexia, proprioceptive disturbances, inability to ambulate, tremor of the tongue and hands, and fluctuations in level of consciousness. Severely ill patients bleed from the mucous membranes and develop shock, anuria, seizures, and coma. Untreated, 15% to 30% die [16,108].

Diagnosis is made in the first few days of the illness by isolating the virus from blood, throat swabs, or urine in biosafety level 4 laboratory. Junin virus antigen and IgM and IgG antibodies may be detected by ELISA testing [16]. Real-time PCR assays are under development [106].

Treatment and prophylaxis are the same as for Lassa fever [14,16,17,84,95]. Convalescent serum therapy may also be effective [97]. Cationic peptides (cecropin A, melittin, and indolicidin) are newer agents that seem to have activity against Junin virus and herpes simplex I and II. Indolicidin inactivated cell-free Junin virus and cecropin A are active against the arenaviruses Tacaribe and Pichinde [109].

Rodent control in or near dwellings is important. A live-attenuated vaccine is available with increasing human safety data, animal efficacy data, but insufficient human efficacy and safety data to support Food and Drug Administration licensure. Unfortunately, the vaccine is only available in Argentina [14,15,110]. Candid #1 vaccine is manufactured in Argentina and in the United States. A 1998 study found vaccine manufactured in the United States to be safe and efficacious [110]. The Argentinean vaccine has been found to be equally safe, immunogenic, and protective in guinea pigs as the vaccine manufactured in the United States [111]. Oils from aromatic plants native to San Luis Province, Argentina, exhibited antiviral activity. *Lippia junelliana* and *L turbinata* were the most potent [112].

Machupo virus: Bolivian hemorrhagic fever

Bolivian hemorrhagic fever is only naturally endemic in the Beni region of northeast Bolivia. The natural host is the rodent *C callosus*. Incubation period is 7 to 14 days and mortality is 10% to 16% [16,17,95]. Treatment and prophylaxis are the same as for Lassa fever [16,17,95]. Convalescent serum therapy may also be effective [97]. Diagnosis is made by isolating the virus in a biosafety level 4 laboratory. Real-time PCR assays are under development [106]. Rodent control in or near dwellings is important. There is evidence of cross-protection by Junin virus vaccine [84,97].

Whitewater Arroyo virus: Whitewater Arroyo virus hemorrhagic fever

The virus for Whitewater Arroyo virus hemorrhagic fever was first isolated from the white-throated wood rat *Neotoma albigula* in northwestern New Mexico. Infection (in mice) results in lymphocytic meningitis and perivascular lymphocytic cuffing. Neonatal infection in the mouse results in chronic (lifelong) infection and viral shedding [113,114]. There have been only a handful of cases.

New therapeutic modalities for Arenaviridae and other RNA viruses

Because RNA viruses replicate with high error rates, they have the potential to rapidly develop drug-resistant mutants. Rather than “chase” the resistant strains with newer anti-infective agents, Grande-Perez and coworkers and others [115,116] attempted to increase the replication error rate to force viral extinction before resistance mutants arise. They did this in vitro (cell cultures) with lymphocytic choriomeningitis virus treated with fluorouracil. The phenomenon was seen at both high and low doses of the mutagen.

*The Bunyaviridae**Bunyavirus genus*

In California encephalitis, La Crosse virus, Jamestown Canyon virus, and Oropouche virus there is encephalitis and generalized febrile illnesses usually without hemorrhagic fever. Bunyamwera virus (previously referred to as Garissa virus) was isolated from two patients and serologically detected in 27% of the patients during a large outbreak of hemorrhagic fever (1997–1998) in Kenya, Tanzania, and Somalia [117]. Studies of the two isolates found that there was genetic reassortment that altered its potential for disease. The Ngari virus was responsible for this outbreak. The Garissa virus is an isolate of Ngari virus, which is a Bunyamwera virus reassortment species [117,118].

Phlebovirus genus: Rift Valley fever

Rift Valley fever virus is a zoonosis that commonly causes abortions in livestock herds. Mortality in ruminants is 10% to 30%, and 1% in humans [119]. Outbreaks are associated with heavy rainfall, sustained flooding, and the appearance of large numbers of mosquitoes [120]. Humans become infected by mosquito bite or handling infected animal blood or tissue. Although human-to-human transmission has not been reported, appropriate infection control procedures should be used. After a 2- to 6-day incubation period, most patients develop a self-limited nonspecific febrile illness [121]. Patients present with sudden onset of fever, headache, joint and muscle pains, conjunctivitis, and photophobia. In a few patients, after a brief initial illness and partial recovery, symptoms return followed by a protracted convalescence.

Five percent to 10% of patients develop retinal disease 1 to 3 weeks after the febrile illness. Patients experience macular exudates, retinal hemorrhages, and vasculitis. Half of these patients have permanent visual impairment. One percent to 5% develops central nervous system complications. One percent develops typical VHF [16,122].

Aggressive supportive care and ribavirin (in similar doses as for Lassa fever) are recommended therapies for complicated cases. Human immune serum is also recommended when available [16,17,121].

Virus may be isolated from the blood during the first week of illness by intracerebral injection into baby mice. Laboratory-acquired cases have

been reported and viral isolation should be performed in a biosafety 4 level laboratory. RT-PCR detects virus in the blood and enzyme immunoassay detects IgG and IGM [121].

Treatment and prophylaxis are the same as for Lassa fever [14,16,17,49,84,95]. A live attenuated vaccine is available for livestock. A formalin-killed Rift Valley fever vaccine is available for humans, but requires an annual booster [122]. Mosquito and rodent control, insect repellants, and personal protective clothing are essential for preventing illness [49,79]. Attenuated mutagenised (by 5-fluorouracil) Rift Valley fever virus vaccine (RFV MP 12) has been tested in sheep, cattle, and Rhesus macaques [122–124]. The South African vaccine strain of lumpy skin disease virus (type SA-Neethling) is being tested for recombinant vaccines expressing the structural glycoprotein gene of bovine ephemeral fever virus or two glycoprotein genes of Rift Valley fever [125]. DNA vaccines for Rift Valley fever, CCHF, tick-borne encephalitis, and Hantaan virus were tested in mice. The Rift Valley fever and tick-borne encephalitis vaccines were protective, whereas the other two vaccines were less immunogenic [126].

Nairovirus genus: Congo-Crimean hemorrhagic fever

The first cases recognized in modern times were documented in 1944. The virus was isolated in 1967 from patients with the Crimean disease and was found to be identical with the virus isolated from a child from the Congo in 1956. Humans are infected by (*Hyalomma* sp) tick-bite, crushing infected ticks against their skin, contact with blood from infected livestock, or from infected patients blood and body fluids [16,17,127]. In some areas, the disease exhibits biannual peaks (March–May and August–October) [128].

The incubation following a tick bite is 1 to 3 days; the incubation following contact with contaminated blood is 5 to 6 days. Nosocomial transmission has been well documented [129]. Most infections are symptomatic. Patients present with sudden onset of fever, chills, headache, dizziness, neck pain, and myalgia. Lymphadenopathy and tender hepatomegaly is seen in most patients. Nausea, vomiting, neuropsychiatric symptoms, and cardiovascular signs and symptoms manifest themselves in some patients as the disease progresses [127]. Some patients develop nausea, vomiting, and diarrhea [130]. Patients later develop flushing and hemorrhage, especially profuse gastrointestinal bleeding. Severely ill patients develop DIC, and renal, hepatic, and respiratory failure [16]. There is a 30% overall mortality [127].

Laboratory studies reveal leucopenia, thrombocytopenia, and elevated transaminase levels. Mortality ranges from 15% to 30% [16,17].

Treatment consists of supportive therapy and ribavirin using the same regimen as outlined for Lassa fever or World Health Organization oral regimen: 30 mg/kg loading dose followed by 15 mg/kg every 6 hours for 4 days, followed by 7.5 mg/kg every 6 hours for 6 days [49,84,95]. This therapy is not approved by the Food and Drug Administration [127].

Although CCHF is transmissible by aerosol, technical difficulties stand in the way of mass production [14,131]. Control of the disease depends on tick control (spraying camp sites, clothing, and tick-infested areas with acaricide or repellents); ribavirin prophylaxis; effective disinfection; and decontamination. Patients must be cared for in strict isolation. Written procedures for the establishment of an isolation unit, barrier nursing, safety measures, handling of patient specimens, and laboratory protocols must be established and enforced [132,133]. Early recognition of disease is essential to limit spread and prevent mortality [134].

Hantavirus genus

Hemorrhagic fever with renal syndrome. During the Korean War (1950–1952), 3000 United Nations troops developed a symptom complex of fever, hemorrhage, acute renal failure, shock, and a mortality of 10% [16]. The etiologic agent (Hantaan virus) was isolated in 1978 from the field mouse, *A agrarius*. Related viruses are the Dobrava virus (the Balkans), which causes severe hemorrhagic fever; Seoul virus (the Far East), which causes a milder hemorrhagic fever; and Puumala virus (Scandinavia), which causes nephropathia epidemica. Puumala virus has also been implicated in cases of HFRS [16,135].

The usual incubation period is 2 to 3 weeks, but may range from 2 days to 2 months. Distinct clinical disease phases have been described [16,136].

1. Febrile phase: Patients present acutely with a flu-like illness with low back pain from retroperitoneal edema, flushing of the face, and conjunctival and pharyngeal injection secondary to vascular dilatation.
2. Hypotensive phase: The second phase consists of mild hypotension or shock and hemorrhage lasting for 1 to 2 days. Patients may only manifest petechiae or develop massive gastrointestinal bleeding or low-grade DIC.
3. Oliguric phase: The third phase is associated with hypertension and renal failure, and in some patients pulmonary edema and change in mental status. If untreated, 50% of the deaths occur at this time.
4. Diuretic phase: The diuretic phase may last for several months.
5. Convalescent phase

Laboratory findings include thrombocytopenia, leukocytosis, hemoconcentration, abnormal clotting profile, and proteinuria. Mortality is estimated at 1%, to 5% to 15% [16,17].

Puumala and Dobrava viruses principally attack the kidneys. All clinically ill patients had enlarged kidneys by sonography. The minority of patients manifest ascites (32% of Dobrava virus infections and 4% of Puumala virus infections) and pleural effusion (16% of Dobrava virus infections). Dobrava virus-infected patients tended to be more ill. They had higher serum creatinine and urea nitrogen levels, 28% required dialysis, and 4% died [137].

An 18-year survey in the Pomurje region of Slovenia found that most cases of HFRS occurred between May and August, and in patients with outdoor occupations. The median age was 39 years, and 19 of the 25 patients were male. Puumala virus caused many more cases than Dobrava virus (23 versus 2 cases). Oliguric renal failure was seen in 13 (57%) of 23 Puumala virus-infected patients. Six (26%) of the 23 patients with Puumala virus infection and one (50%) of the two patients with Dobrava virus infection were hypotensive with signs of shock. Seven (47%) of 15 patients had elevated cerebrospinal fluid protein (all patients), and 7 (41%) of 17 Puumala virus patients had sinus bradycardia [135].

Seoul virus produces milder disease with febrile, mild hemorrhagic manifestations, with hepatomegaly and mildly elevated liver enzymes. Puumala virus produces the mildest infection with fever, petechiae, and the onset of oliguria on Day 6 of disease. Ten percent of patients require dialysis, and 20% develop reversible central nervous system symptoms (confusion and dizziness) [16,17].

A recent outbreak of HFRS among Russian military personnel stationed in the Primorskii region reported the greatest number of cases within 2 weeks of the first case. There were 104 cases; 77.8% were 18 to 20 year olds; and there was 7.4% mortality [138].

A study of 1600 Korean War veterans who had contracted HFRS found no increase in long-term mortality and only a questionable increase in selective morbidities [139]. Hypopituitarism with an atrophic pituitary gland and an empty sella (on MRI) has been reported as a late complication of HFRS [140].

Aggressive supportive therapy and ribavirin has been shown significantly to improve survival. A killed vaccine is available with increasing human safety data, animal efficacy data, but insufficient human efficacy and safety data to support Food and Drug Administration licensure [84]. Formalin inactivated vaccines for Hantaan and Seoul viruses have been used in Asia [16,141]. Chinese monovalent vaccines are said to be 95% effective. The Chinese have data on a new bivalent vaccine that seroconverts 85% of patients with an adverse reaction rate of 0.5% [141].

Hepatitis B virus core particles with the amino-terminal 120 amino acids of the nucleocapsid of Dobrava, Hantaan, or Puumala viruses (chimeric core particles) were found to be highly immunogenic in BALB/c and C57BL/6 mice [142]. Another candidate vaccine is the recombinant *Peromyscus maniculatus* cytomegalovirus vaccine that contains Sin Nombre virus glycoprotein GI fused to enhanced green fluorescent protein gene [143].

Limiting human exposure to rodents and excreta is essential for prevention of this disease [16]. There are two studies that suggest that person-to-person spread of hantavirus of the type that causes HPS [144,145] is unlikely in the hospital setting and one study that demonstrates the possibility [22]. The differential diagnosis includes leptospirosis, typhus, pyelonephritis, poststreptococcal glomerulonephritis, acute surgical abdomen, and other

hemorrhagic fevers [16,17]. Although HFRS is transmissible by aerosol, technical difficulties stand in the way of virus mass production [14].

Hanta virus pulmonary syndrome: Sin Nombre virus. HPS incubation period typically is 1 to 2 weeks but ranges from 1 to 4 weeks. Prodromal stage is usually 3 to 5 days (range: 1–10 days). Patients have an abrupt onset of symptoms: fever, myalgia, malaise, chills, anorexia, and headache. As the patient worsens, there is prostration and significant nausea, vomiting, abdominal pain, and diarrhea. Some patients present with only mild to moderate generalized discomfort. In the cardiorespiratory compromise stage, patients initially are short of breath and have evidence of pulmonary edema. They have a productive or nonproductive cough, tachypnea, fever, mild hypotension, and arterial oxygen desaturation.

Chest radiographs may be initially normal, but progressively worsen, displaying signs of pulmonary edema and acute respiratory distress syndrome. Other laboratory abnormalities include thrombocytopenia, leukocytosis, atypical lymphocytes, mildly elevated aspartate transaminase serum, prolonged partial thromboplastin time, increased serum lactate dehydrogenase, and lactic acidosis. Few patients develop DIC and bleeding.

Patients deteriorate at different rates, some dying rapidly, whereas others deteriorate and desaturate more slowly. Most deaths occur within 48 hours of admission. One third of the patients are managed successfully, and if patients survive the first 2 to 3 days, they will probably recover.

Independent of the pulmonary pathology, patients are found to have low cardiac outputs, elevated systemic vascular resistance, and normal to low pulmonary wedge pressures. Patients succumb to fatal shock and lactic acidosis. Those who survive without complications of therapy are often discharged in 2 weeks [18]. Renal failure requiring hemodialysis has accompanied acute infection [146]. Ribavirin is apparently ineffective. Intensive care decreased the mortality and a vigorous neutralizing antibody response seems to be predictive of survival [147]. Serologic testing for Sin Nombre virus may be accomplished by ELISA or Western blot assays [148].

The Filoviridae

Ebola virus: Ebola hemorrhagic fever

Following a 4- to 10-day (range: 2–21 days) incubation period, patients present with an abrupt onset of fever, severe headaches, myalgia, abdominal pain, diarrhea, and pharyngitis, with herpetic-like lesions on the mouth and pharynx. There is severe conjunctival injection and bleeding from the gums. Light-skinned patients often have a prominent maculopapular rash, which evolves into petechiae; ecchymosis; and bleeding from venepuncture sites and mucosa (with hematemesis, bloody diarrhea, and generalized mucosal hemorrhage). Neurologic complications (hemiplegia,

psychosis, coma, and seizures) are common. Patients go on to develop shock, metabolic acidosis, and diffuse coagulopathy. Patients succumb by Day 10 with mortality rates from 60% to 90% for Ebola Zaire and 50% to 60% for Ebola Sudan. Mortality rates are higher for those infected by contaminated needles [16,17,65].

Initial leucopenia and lymphopenia is replaced by increases in the white blood cell counts and the appearance of viral-infected large abnormal lymphocytes with dark cytoplasm (virocytes) [16]. Although the clinical presentation for both viral infections seems to be severe disease, serosurveys in equatorial Africa in the mid-1990s found a 20% seropositivity rate for filoviruses in some populations. This suggests that the diseases maybe be widespread, and the most common infections are mild or asymptomatic [65].

Differential diagnosis is the same as for Lassa fever. Diagnosis is made in the first few days of the illness by isolating the virus in Vero cells in biosafety level 4 laboratory. Antigen may be detected by RT-PCR and antigen-capture ELISA. IgM and IgG antibodies may be detected by ELISA testing [16].

Strict isolation, safe burial practices, and an active surveillance system are described [149]. Presently, there is no vaccine or therapy available for Marburg or Ebola virus hemorrhagic fevers. Filovirus matrix protein VP40 drives spontaneous production and release of virus-like particles that resemble the infectious virions. Addition of other filovirus proteins (VP24, VP30, VP35, and filovirus glycoprotein) increases the virus-like particles production and particles that express multiple filovirus antigens. Injection of rodents with (Ebola or Marburg) virus-like particles containing glycoprotein and VP40 protects them from lethal challenges with Ebola and Marburg viruses [150–152].

Cytotoxic T lymphocytes are essential for survival during an Ebola virus infection. In C57BL/6 mice, vaccination with Venezuelan equine encephalitis virus replicons encoding for Ebola virus nucleoprotein survived a normally lethal Ebola virus infection. Polyclonal antiserum against Ebola virus nucleoprotein was not protective, whereas transfer of cytotoxic T lymphocytes specific for Ebola virus nucleoprotein was protective against Ebola virus infection [153].

Vaccines against Ebola virus are particularly difficult to develop because the disease observed in primates differs from the disease in rodents. Work with the unsuccessful vaccine candidates (attenuated Venezuelan equine encephalitis virus expressing Ebola glycoprotein and nucleoprotein; recombinant vaccinia virus expressing Ebola glycoprotein; liposomes containing lipid A and inactivated Ebola virus; and concentrated inactivated whole-virion preparation) demonstrated protection against Ebola for mice and guinea pigs, but failed in nonhuman primates [154]. Vaccine against Ebola and Marburg viruses based on recombinant vesicular stomatitis virus has been shown to protect macaques from lethal doses of the corresponding filovirus [155,156].

Prediction of epidemic spread of Ebola has been attempted by monitoring the spread of disease in nonhuman primates. Ebola outbreaks in the

forest zone between Gabon and the Republic of Congo (2001–2003) were the result of handling wild animals (carcasses) that had died of infection. A monitoring system collected 98 carcasses of which analysis of 21 found that 10 gorillas, 3 chimpanzees, and 1 duiker were seropositive for Ebola virus. Between 2001 and 2003, there were five human Ebola outbreaks, each preceded by an animal zootic. The surveillance method was able to alert the health departments of two of the outbreaks weeks in advance [157].

Marburg virus: Marburg hemorrhagic fever

The clinical presentation of Marburg virus infection is similar to that of Ebola virus. The incubation period is 3 to 10 days after which there is a sudden onset of fever, chills, headache, and myalgia. After 6 to 8 days the illness may progress to severe hemorrhagic fever. On the fifth day of illness, some patients develop a maculopapular rash followed by nausea, vomiting, chest pain, sore throat, abdominal pain, and diarrhea. As patients become worse, jaundice, pancreatitis, weight loss, delirium, shock, liver failure, massive bleeding, and multiorgan failure develops [158].

Although the mortality has been recorded as 25% to 90%, it is closer to 25% to 30%. Virus may be isolated from survivors' semen as long as 3 months after recovery. Uveitis with viral isolation from the anterior chamber has been reported [16,17].

Diagnosis is made by isolating the virus in biosafety level 4 laboratory. Antigen may be detected by PCR and antigen-capture ELISA. IgM and IgG antibodies may persist for long periods [16].

An effective public health response (as seen during the outbreak in Angola, 2005) involves (1) accurate diagnosis, (2) isolation of patients and contacts, and (3) proper infection control procedures at health care facilities. Strict isolation precautions for patients are absolutely necessary. There is no vaccine or specific antiviral therapy presently available [158,159].

The Flaviviridae

Dengue and dengue hemorrhagic fever

This is the most common VHF with some estimates of over 100 million cases per year worldwide. The disease is found in every tropical country and is principally spread by *A aegypti*. There are four serotypes that produce three clinical syndromes: (1) nonspecific febrile illness; (2) dengue fever (fever, arthralgia, and rash); and (3) dengue hemorrhagic fever.

Classical dengue. The incubation period for dengue is 2 to 7 days. Patients develop “break bone fever,” a sudden onset of high fevers, severe muscle pains, headache, and prostration with facial flushing, retro-orbital pain, and conjunctival injection. Half the patients have an early transient erythematous rash. Patients remain febrile, anorexic, and restless for 4 to 6 days and display mild hemorrhagic signs (positive tourniquet test,

epistaxis, petechiae, or purpura). Platelet counts are usually above 100,000/mm³, but may drop to low levels with the white blood cell count. The fever then rapidly resolves and a morbilliform or scarlatiniform rash appears, first on the extremities (with petechiae on the legs) along with generalized lymphadenopathy. Liver enzymes are abnormal but usually there is no hepatomegaly. There is a second febrile phase lasting 2 to 3 days followed by a desquamation of the rash. Convalescence is long, and patients remain debilitated and depressed during this period [16,17,121].

Dengue hemorrhagic fever. Dengue hemorrhagic fever begins on Day 2 to 5 of the classical illness. At this time, the patient goes into shock and exhibits restlessness; diaphoresis; hypotension; and hemorrhagic manifestations (positive tourniquet test, petechiae, purpura, spontaneous bleeding from the gums and gastrointestinal tract). The liver becomes enlarged and tender and some patients develop hypoproteinemia, hyponatremia, and mild elevations of the liver enzymes. Thrombocytopenia, sometimes below 10,000, develops. There is increased capillary permeability with hemoconcentration (hematocrit increasing $\leq 20\%$), DIC, and leucopenia, and patients develop hypotension (dengue shock syndrome). Respiratory failure ensues from alveolar hemorrhage and fluid accumulation. Renal failure secondary to hypotension and immune complex deposition, and rarely encephalopathy, follows. Mortality is 10% for dengue hemorrhagic fever and may be reduced to $< 1\%$ with adequate fluid resuscitation [16,121].

Virus may be detected early in the disease by growth on cell culture or RNA detected by PCR. Serologic tests are also available (hemagglutination inhibition test, enzyme immunoassay, or immunofluorescence assay) on acute and convalescent serum samples [16,121].

There is no specific antiviral therapy. Aggressive supportive therapy is necessary. Candidate vaccines and chemotherapeutic agents have been discussed (see Yellow fever next). Strict isolation except for the exclusion of mosquitoes is not necessary [16,121].

Ampligen (polyI:polyC12U) is a mismatched double-stranded RNA that induces interferon production. It is being investigated for therapy of chronic fatigue syndrome; HIV infection; Epstein-Barr virus-positive Hodgkin's lymphoma; severe acute respiratory syndrome; hepatitis C; renal cell carcinoma; invasive or metastatic malignant melanoma; immune dysfunction syndrome; and flaviviruses (West Nile virus, equine encephalitis virus, dengue fever virus, and Japanese encephalitis virus). Hemisphere Biopharma has an oral Ampligen-like (polyI-polyC126) compound available, that could possibly be of great value in a mass-casualty setting [160].

A live-attenuated vaccine is available with increasing human safety data, animal efficacy data, but insufficient human efficacy and safety data to support Food and Drug Administration licensure [84]. Some authorities do not consider dengue a bioterrorism risk because primary dengue rarely causes hemorrhagic fever, and it does not seem to be transmissible by small particle

inhalation [14,131]. Others, however, think otherwise. As an example, Dr. Kamal Datta, director of the National Institute of Communicable Diseases (India), referred to the outbreak of dengue in Delhi in 1996 (10,252 cases; 423 deaths) as “suspicious” [161].

Yellow fever

Yellow fever is found in tropical Africa and South and Central America. In the nineteenth and early twentieth century, it was also endemic in temperate areas as far north as Philadelphia. The virus is maintained in *Haemagogus* and *Sabethes* mosquitoes in forests, and *A aegypti* are usually responsible for outbreaks in population centers. In Africa, there are monkey-mosquito-monkey cycles involving other *Aedes* species. In Brazil, the *Amblyomma variegatum* tick transmits the virus transstadially and passes the virus to uninfected monkeys.

The incubation period is 3 to 6 days. In endemic areas, many have unapparent infections with significant immunity. Most symptomatic infections are mild with patients recovering within 48 hours or less. The minority of patients has severe headache, myalgia, low back pain, and proteinuria. Patients exhibit a relative bradycardia for the degree of fever (Faget’s sign).

Patients with severe illness have abrupt onset of high fever; severe headache; nausea, vomiting; and abdominal, back, loins, and limb pain. Patients become dehydrated, jaundiced, and develop bleeding from the nose and gums. This “period of infection” (viremia) last for 3 days. Patients may enter a recovery phase or temporarily improve over the next 24 hours and then rapidly deteriorate (jaundice deepens and patients develop epigastric pain, vomiting, and gastrointestinal bleeding). Patients become hypotensive and develop heart failure and prolongation of the PR and QT intervals on EKG. Patients may recover over 3 to 4 days to 2 weeks, or death may occur on the seventh to tenth day.

Complications include parotitis and pneumonia. Increasing proteinuria, bleeding, tachycardia, oliguria, renal insufficiency, and hypotension are all bad prognostic signs. Mortality is 20% to 50% [16,121].

Virus may be isolated from the blood in the first 3 days. Other methods of diagnosis are antigen-capture enzyme immunoassay; RT-PCR; or on liver biopsy specimen by immunofluorescence assay, probe hybridization, or RT-PCR [121]. A licensed live-attenuated vaccine is available [85].

Kyasanur Forest disease virus and hemorrhagic fever

Kyasanur Forest disease is a tick-borne (*Haemaphysalis* ticks) flavivirus of southwestern India. There is a 3- to 8-day incubation period. It produces a febrile or hemorrhagic disease. A vaccine is available and infection control methods include treatment of cows for tick infestations and the use of insect repellents. Mortality is 3% to 10% [16,17]. Asymptomatic infection probably occurs because serology studies (2401 samples from six locations on the Andaman and Nicobar Islands of India) found 22.4% antibody prevalence [162].

Because of its potential use as a bioterrorist weapon, this virus requires a biosafety level 4 laboratory for isolation [15].

Omsk hemorrhagic fever virus and disease

Dermacentor ticks are the principle reservoir, and the infection is found in many small mammals in Siberia including the muskrat. Human infection is acquired by either handling dead carcasses or by tick bites. Infected muskrat hunters are often asymptomatic. There is a 3- to 8-day incubation period for symptomatic disease. Some develop papulovesicular lesions on the soft palate, and mucosal and gastrointestinal bleeding. There is a 0.5% to 10% mortality in symptomatic cases [16,17].

BALB/c mouse model has been used for Omsk hemorrhagic fever and the neurotropic flavivirus, Powassan virus. Pathologic differences (cerebellar involvement with Powassan virus infection and different splenic pathology) were demonstrated. Omsk viral antigen was seen in the spleen, brain, and endothelial cells of the liver but was absent from the kidneys. Powassan virus was detected in the brain but was absent from the spleen and kidneys. The distribution of the viral particles in the brain differed [163].

Because of its potential use as a bioterrorist weapon, this virus requires a biosafety level 4 laboratory for isolation [15].

Alkhumra virus and hemorrhagic fever

Four pilgrims from the 2001 Hajj to the holy city of Mecca, Saudi Arabia, were identified in February of that year as having typical VHF. A new flavivirus, a close relative to the tick-borne Kyasanur forest disease virus, was the same virus isolated in 1995 from six patients with dengue-like hemorrhagic fever from the Alkhumra district, south of Jeddah, Saudi Arabia [164–167]. Further investigation found two additional cases in 1994, both in butchers and both organisms recovered from wounds [166]. Sources of infection identified in the original cases included contaminated wounds (butchers, six); tick bites (student and engineer, two); and consumption of raw camel milk (soldier, driver, and poultry worker, three) [166]. The investigation identified 37 patients (20 laboratory confirmed). All presented with an acute febrile flu-like illness and hepatitis; half displayed hemorrhagic findings (55%); and 20% had encephalitis. It was concluded that the disease was transmitted by mosquito bite or direct contact with infected sheep, goats, and rodents. How the virus is maintained in nature is under investigation. Of 11 cases identified between 1994 and 1999, four died [166]. Overall, there was 25% mortality with this outbreak [164,165].

Because of its potential use as a bioterrorist weapon, this virus requires a biosafety level 4 laboratory for isolation [15,167].

Summary

Specific pharmacologic therapy (ribavirin) for VHF is recommended for Lassa fever and the other arenavirus hemorrhagic fevers, Rift Valley fever,

CCHF, HFRS, and related viruses. There is no specific antiviral therapy available for flavivirus or filovirus hemorrhagic fevers. The lack of an approved vaccine or proved prophylaxis and survival of many of the most severely ill patients with inpatient intensive care make this group of viruses potential bioterrorism agents with the intention of overwhelming society's resources.

Mobilization is immediately needed for:

1. Vaccine development
2. Specific prophylactic and therapeutic interventions
3. Strengthened public health response including an efficient system to monitor contacts and populations at risk
4. Invigorated hospital infection control practices
5. Effective regional or national triage systems
6. Expansion of inpatient isolation facilities to accommodate intensive levels of care
7. Regionalization of inpatient intensive care facilities to maximize the care that may be delivered to the sickest victims
8. Development of effective and acceptable outpatient therapeutic care

A unique suggestion has been the development of immune response modifiers that stimulate the immune system's antiviral and antineoplastic activity. Coley Pharmaceutical Group, 3M Pharmaceuticals, Hybridon, SciClone, Hemispherx Biopharma, Corixa Corporation, and AFG Biosolutions have products in various stages of development. Hybridon's second-generation immunomodulatory oligonucleotide may be potentially useful for prophylaxis or therapeutically for variola, dengue, and Ebola in addition to other pathogens [168].

Toll-like receptors on immune cells recognize pathogen macromolecules with wide specificity. 3M Pharmaceuticals' Aldara (imiquimod) has been shown to be active against human papillomavirus, molluscum contagiosum, and leishmaniasis, and is approved for the treatment of actinic keratoses, basal cell carcinoma, and genital warts. The drug stimulates toll-like receptor-7 and toll-like receptor-8, which have activity (in the mouse and primate models) against herpes simplex 1 and 2, cytomegalovirus, influenza, Banzivir, Rift Valley fever, and West Nile virus. Coley Pharmaceutical Group's GPG 7909 is a toll-like receptor-9 agonist [168].

With the best defense being prevention, a worldwide inventory of existing viral stocks must be both accurately recorded and secured. Aggressive international profiling and tracking of individuals who acquire or seek to acquire specialized viral laboratory equipment, viral cultures, viral hosts or vectors, and visit viral habitats, or have contact with their natural victims, needs to be undertaken. Such programs deter all but the most determined, resourceful, and elaborately sponsored malefactors. Denying resources to a determined and well-financed enemy protected by a foreign government and its international boundaries is difficult if not impossible. Only intelligence

agencies' clandestine targeting of state and "stateless" organizations is able to address threats from the remainder.

A valuable and frequently updated resource that tabulates the etiologic agents, their characteristics, a resource list, and clinical pathways may be found at the Infectious Diseases Society of America website (<http://www.idsociety.org/>) under "Resources" and "Bioterrorism."

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